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GAMETOGENESIS AND FERTILIZATION IN ALBUGO.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XXIX.

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(WITH PLATES I–IV)

BEFORE the publication of my paper (1899) describing the fertilization of *Albugo Bliti*, the mature oosphere in the Phycomycetes was generally conceded to be a uninucleate structure. The question of homologies in the group did not then seem difficult. *A. Bliti*, however, presented a unique condition, in that about one hundred antheridial nuclei fuse in pairs with an equal number of egg nuclei. This condition seemed remarkable and inexplicable, inasmuch as Wager (1896) had studied the fertilization in *A. candida*, and had described a fusion of single male and female nuclei in a uninucleate oosphere. Such divergence in the same genus led to some speculation. Davis (1900) reexamined *A. candida* and fully confirmed Wager's account, as indeed Berlese (1898) had already done. It is well established, therefore, that there is a simple fusion in *A. candida*, and my own account (1899) stands for a multiple fusion in *A. Bliti*. Interest also attaches to this genus on account of the presence of a coenocentrum, a structure of prominence, but of hitherto unknown function.

The study of *A. Portulacae* and *A. Tragopogonis* was undertaken in the hope that these species might help to explain the

apparent discrepancy between the two species previously studied. The selection of *A. Tragopogonis* has proved particularly fortunate, for it has led directly to a solution of the problem. The conditions in this species, while to a certain extent similar to those described for *A. candida*, made evident the necessity of a critical reexamination of the latter. I have included *A. candida*, therefore, in this investigation.

The material of *A. Portulacae* on *Portulaca oleracea*, and *A. Tragopogonis* on cultivated *Tragopogon* and *Ambrosia artemisaefolia*, was collected at Syracuse, New York. The material of *A. candida* on *Bursa* was collected at Columbus, Ohio. All the material was fixed in chrom-acetic killing fluid and imbedded in paraffin in the usual way. The methods in general were the same as those employed in the study of *A. Bliti*, and are described in detail in BOT. GAZ. **28**: 233. 1899. The Flemming triple stain was used throughout, and I am convinced that it is best for the study of Albugo, although it is exceedingly sensitive to manipulation, and the different species of Albugo require quite different treatment.

The results of this investigation are presented in three sections, entitled "descriptive," "phylogeny," and "general considerations." The first section describes the phenomena observed, avoiding theoretical considerations and presenting only what may be regarded as established facts. The second section considers the phylogenetic bearing of this research. The third section considers the genus from the comparative standpoint, an attempt being made to interpret the significance of the phenomena, and to call attention to analogous phenomena in other organisms.

The species discussed were kindly determined by Dr. Paul Magnus, who assures me that the names and synonymy are as follows: ALBUGO PORTULACAE (DC.) O. Ktz. (*Uredo Portulacae* DC., *Cystopus Portulacae* Lév.); A. TRAGOPOGONIS (Pers.) S. F. Gray (*Uredo candidus Tragopogonis* Pers., *Cystopus Tragopogonis* Schröt., *Uredo Tragopogonis* DC., *Cystopus cubicus* Lév., *C. spinulosus* DBy., *Uredo cubica* Strauss); A. CANDIDA (Pers.) O. Ktz. (*Uredo candidus* Pers.).

I wish to express my chief thanks to Professor Strasburger for daily counsel during the progress of this research; to Professor Magnus for names and synonymy; to my wife for the preparation of the slides, and for Plate IV; and to the University of Chicago for the advantages derived from a traveling fellowship.

I. DESCRIPTIVE.

ALBUGO PORTULACAE.

The early stages of the sex organs in *A. Portulacae* differ in no essential detail from those described for *A. Bliti* (Stevens 1899, *figs. 42, 43, 45, 59–65*). The nuclei are distorted as the protoplasm flows into the developing oogonium, but as this structure attains its full growth and recovers its turgor they regain their spherical form and enlarge, rapidly assuming the spirem condition. They are more numerous than in any of the other species examined, ranging from 300 to 400 in each oogonium, and are smaller than in *A. Bliti*, rendering this an unfavorable type for cytological study.

The aggregation of the cytoplasm into several regions of greater density is the first indication of the development of the oosphere (*fig. 1*). The aggregations soon coalesce, forming one large mass of fine uniform cytoplasm. Apart from the structural differences between the dense alveolar center and the vacuolate filar periphery, there is a very distinct difference in stain reaction. The dense fine-grained cytoplasm refuses the gentian, but takes the orange G lightly, while the vacuolate peripheral cytoplasm takes the gentian strongly. The uniform dense alveolar region is the rudimentary oosphere, and is centrally placed in the oogonium (*fig. 3*).

Throughout this differentiating process the nuclei, which are now in mitosis, are crowded out of the denser masses and come to lie near the larger vacuoles (*fig. 2*). Therefore, after the denser masses have coalesced and the larger vacuoles are forced into the periplasm, the nuclei are to be found near the rather indefinite boundary between periplasm and ooplasm. This boundary, however, becomes distinct and sharp immediately

after the nuclei have passed to the periphery. Meanwhile the mitosis advances from prophase to metaphase.

The oogonium in this condition presents typically a region of uniform finely vacuolate cytoplasm devoid of nuclei, surrounded by a zone of cytoplasm bearing large vacuoles and containing the nuclei in metaphase. This condition I have termed the stage of *zonation* (*fig. 3*). A full understanding of this stage is necessary to interpret it in other species. Zonation presents the first clear differentiation of ooplasm from periplasm at a time previous to the existence of any wall between these parts, and finds the ooplasm nearly or quite devoid of nuclei. Zonation is very definitely and clearly marked in *A. Bliti* and *A. Portulacae*, the periplasm and ooplasm being as sharply separated as though an actual wall existed between them, but is much less conspicuous in *A. Tragopogonis* and *A. candida*, thus rendering these species more difficult of interpretation.

Immediately following zonation the nuclei divide, many of them lying in such a position that one of their daughter nuclei reenters the ooplasm. In *A. Bliti* nuclei are frequently found in late anaphase with one daughter nucleus lying inside of the oosphere and the other in the periplasm, thus affording direct evidence of the derivation of the primary oospheric nuclei. This rarely occurs in *A. Portulacae*, as the dividing nuclei usually lie quite outside of the line separating ooplasm and periplasm. The results, however, are precisely those presented in *A. Bliti*, namely, one of the daughter nuclei of each mitotic figure, lying with its long axis approximately perpendicular to the boundary of the oosphere, reenters the ooplasm (*figs. 4, 5*). The resulting oosphere contains many nuclei, the number usually varying from 50 to 100 in this species.

These primary oospheric nuclei now divide mitotically, and their products function as the female sexual elements. It should be carefully noted that there are here, as in *A. Bliti*, two mitoses in the oogonium. The first occurs during the differentiation of the oosphere and provides the primary oospheric nuclei. The second occurs in the oosphere (*figs. 6, 7*) and results in the

female nuclei. These two divisions may be recognized at a glance, inasmuch as the mitoses are simultaneous for all the nuclei concerned. The nuclear figure of the second mitosis differs from that of the first in that the nuclear membrane disappears and there are fewer spindle fibers. The second mitosis usually affects only those nuclei that lie in the clearly differentiated oosphere.

Peculiar activities are present in the center of the oogonium shortly before zonation, which lead to the formation of the coenocentrum. This structure, much less conspicuous and much more ephemeral in *A. Portulacae* than in *A. Bliti*, is of the same general nature, however, and needs no special description. The central globule is found only rarely and is very small.

The development of the antheridium of *A. Portulacae* is similar to that of *A. Bliti* in its general features. Simultaneously with the mitosis of the oogonium two mitotic divisions occur in the antheridium, but the difference which is so clearly apparent between the nuclear figures of the first and second mitoses in the oogonium is not found in the antheridium. The antheridial cytoplasm stains darkly with the gentian violet, and resembles the periplasm rather than the ooplasm of the oogonium. The antheridia lie closely appressed to the oogonia, and it is not unusual to find more than one adhering to the same oogonium, a condition much more rarely seen in *A. Bliti*.

A slight papilla is developed from the oogonium into the antheridium at their point of contact. This is the "receptive papilla," first described by Wager (1896) for *A. candida*, and it reaches most remarkable proportions in *A. Portulacae* (*figs. 1, 5, 6*). In its early stages it resembles the receptive papilla of *A. candida* and *A. Bliti*, being merely a slight protuberance extending from the oogonium to the antheridium.[1] In *A. Portulacae*, however, it reaches much greater proportions (*fig. 5*). Its development occurs at a later period than in the other species, usually after the differentiation of the oosphere or even during

[1] The account of the structure in *A. Bliti* (Stevens 1899) traces the growth of the papilla in detail.

the second mitosis, while in *A. Bliti* it precedes the preliminary massing of the cytoplasm. In *A. Portulacae* the oosphere sometimes protrudes into this papilla, a phenomenon not occurring in *A. Bliti*, since its oosphere has not been differentiated when the papilla is formed. The condition presented in *fig. 6* shows that sometimes the papilla becomes ruptured. This I cannot regard as normal, however, since only one of the hundreds of preparations examined shows such a condition.

The mode of formation of the antheridial tube is uncertain. In somewhat advanced stages it is found extending nearly to the center of the oosphere. It has a well-defined wall, and is firm and straight (*fig. 8*), differing from the swollen gelatinous tube of *A. Tragopogonis* and *A. candida*, but agreeing closely with that of *A. Bliti*.

In no case was the antheridial tube in a mature oogonium seen to contain less than fifteen nuclei, and in most cases there are probably 150 or more. I have seen the tubes in transverse, longitudinal, and oblique sections, and they were always multinucleate and extended into multinucleate oospheres (*figs. 7–9*). The male nuclei are oval as they lie in the end of the antheridial tube, and stain more darkly at the anterior end. Whether there is any constant numerical relation between the sperm nuclei and oospheric nuclei could not be determined with any accuracy, owing to the great number of sperm nuclei crowded into the antheridial tube. When fully developed, the antheridial tube opens as in *A. Bliti*. There is first a softening of the wall, which is probably due to the presence of an enzyme, and it is finally dissolved, allowing the nuclei to escape and pair with those of the oosphere (*figs. 10, 11*). Before fusion both male and female nuclei enlarge somewhat, although they maintain a typical resting condition during fusion and regain their original size soon after. The result is an oospore containing between 100 and 200 nuclei, which passes the winter without further change.

A very few winter spores were seen with a relatively small number of nuclei, in one case as few as six, but this condition must be regarded as very exceptional. Such cases probably

result from a fertilization of oospheres containing few female nuclei, and such are rare.

It is unnecessary to describe further the development of the spore, since its course is apparently parallel with that of *A. Bliti*. It consists simply of an accumulation of food stuffs and a growth of protective structures. A portion of a mature wall is shown in *fig. 12*. The endospore consists of one layer only, not two, as I have described in *A. Bliti*.

Berlese, in a recent paper, entitled "Ueber die Befruchtung und Entwickelung der Oosphäre bei den Peronosporeen," discusses some of the cytological phenomena of this genus. In this study he has included observations on *A. Portulacae*, giving six figures to represent the species. His results differ essentially from mine, a difference which is the more interesting since similar technique was employed, and in both cases the host plant was *Portulaca oleracea*. For the sake of clearness I will indicate some of the discrepancies between his work and my own. Berlese finds thirty to forty nuclei in an oogonium before mitosis, and says that these nuclei divide several times, the nucleoli vanishing during prophase. My preparations show 300 to 400 nuclei which divide twice, their nucleoli persisting till late anaphase. He finds ten to twelve nuclei in an antheridium, while my material shows a number several times greater. Notwithstanding his statement (p. 176), "das Cytoplasma, welches den in der Gonosphäre zurückgebliebenen Kern umgiebt, ist dicht und sehr fein gekörnt," the ooplasm which he represents is much more coarsely vacuolate (Berlese, 1898, *figs. 2–4*) than in any Albugo I have examined. Indeed, it does not resemble the ooplasm of a typical Albugo, which varies but little, so far as my observation goes, but rather presents the features of the ooplasm of Peronospora. He notes no "receptive papilla," and the antheridial tube in both structure and content is very unlike that described in this paper. He describes the chromosomes as visible in the male nucleus during fusion (p. 179), and counts them. None of the species I have examined presents a possibility of counting chromosomes at this stage, inasmuch as the

nuclei fuse in typical resting condition, and the chromosomes are then absolutely indistinguishable. Many other discrepancies might be enumerated.

Such marked divergences, aside from the fact that Berlese has described a uninucleate oosphere and antheridial tube, while I find them both clearly multinucleate, are sufficient to warrant the suspicion that we are working with quite different species. Still, only one species is as yet described on *Portulaca oleracea*, and the configuration of the spore wall represented and described by Berlese agrees fully with that of the form discussed in this paper.

I have taken particular care to convince myself of the identity of the species used, and have the assurance of Professor Magnus that my species is truly *Albugo Portulacae*. Furthermore, I have been able to compare this material with authentic European herbarium specimens, and they agree precisely. Not only do my preparations agree with European material in gross characters, but the cytoplasmic structures agree with those carefully figured by Istvánffi (1895, *pl. 36, figs. 24, 25*). This agreement emphasizes the identity of the American with the European species, and further shows that the species from widely separated regions[2] do not vary appreciably even in the cytoplasmic phenomena regarding which Berlese and I report such diametrically opposed observations.

It is difficult to explain these numerous and serious discrepancies. Great variation in cytological detail is to be expected in this genus, and possibly the Italian form differs from the American as well as from that which Istvánffi studied. This is improbable, however, in view of the close agreement between these two latter forms. The problem can hardly find definite solution until the Italian form has been studied more closely. A mere difference in the number of functional nuclei, even a difference so great as that between the multinucleate and uninucleate oosphere, is readily conceivable in the same species when

[2] I have been unable to learn where Istvánffi collected his material, but assume that it is of European origin.

the condition presented by *A. Tragopogonis* is borne in mind. Berlese, however, describes no such phenomena as are seen in *A. Tragopogonis*, nor does he see the coenocentrum. Thus it appears that in the Italian form the reduction to a uninucleate condition, if such a condition really exists, is of an entirely different nature from that described in this paper.

ALBUGO TRAGOPOGONIS.

In *A. Tragopogonis* there is a simple fertilization. One male nucleus is conveyed into the oosphere, where it fuses with one oospheric nucleus. To understand the variation between this form of fertilization, also characteristic of *A. candida*, and that shown by *A. Bliti* and *A. Portulacae*, it is necessary to follow carefully the stages in the development of the oosphere in the two former species, and to homologize them with those presented by the two latter.

In *A. Tragopogonis* the oogonium and oospore are smaller than in *A. Bliti* or in *A. Portulacae*. The number of nuclei is less in proportion to the size of the oogonium, however, so that for each nucleus there is more available space. This obviates much of the dense crowding of mitotic figures seen in *A. Bliti* and in *A. Portulacae* at the time of zonation, rendering this a favorable type for study. The small size of the oosphere, however, makes it much more difficult to recognize the stages of development, so that even the preliminary examination must be made under an immersion lens.

The early stages are similar to those described for *A. Portulacae*. The protoplasm flows into the oogonium, the nuclei enlarge, the cytoplasm masses in the center, and finally the fully developed stage of zonation is reached. As in *A. Bliti* and *A. Portulacae*, this is a well-marked developmental stage (compare *figs. 28* and *3*), although it does not stand out with quite the clearness that obtains in those species. There is one central area of dense fine-meshed alveolar ooplasm, which is very clearly differentiated from the filar deeply staining periplasm. The central region, which at this time contains a very prominent

coenocentrum, is entirely devoid of nuclei. They surround the central region, lying wholly within the periplasm, and are usually in metaphase. In this species, as in *A. Portulacae*, the mitosis is nearly completed in the periplasm, and the daughter nuclei do not begin to push back into the ooplasm until the chromosomes have retreated from the equatorial region of the spindle. It is also apparent that only those nuclear figures which are appropriately oriented contribute daughter nuclei to the ooplasm, and that only one of the pair gains entrance. From their mode of entrance it follows, as in *A. Portulacae*, that the chromatic content of the nuclei is situated at the end most distant from the sister nucleus. The nuclei number about fifty, and the tendency toward lowering the number that is occasionally seen in *A. Portulacae* is not apparent here.

The primary oospheric nuclei now undergo a second mitosis, which is clearly distinguishable from the first by the character of the chromatic figure, which is similar to that described for *A. Portulacae*. The fact that the second mitosis involves only the oospheric, not the periplasmic nuclei also distinguishes it from the first (*figs. 32, 34*).

The definiteness with which ooplasm and periplasm are delimited at and after zonation (*figs. 28–31*) precludes any possibility of confounding pre- and post-zonation stages. The clearness of the nuclei, which are seen as they enter the oosphere (*fig. 29*), and which can be followed through all the stages of the second mitosis (*figs. 32, 34*), renders it equally certain that in *A. Tragopogonis* the oosphere is multinucleate. So far there has been no deviation from the course followed by *A. Bliti* and *A. Portulacae*.

These potential female nuclei appear to differ from each other in no important respect, unless it be in their distance from the coenocentrum, yet under the usual conditions only one of them is destined to function as a sexual nucleus. A study of later stages shows that one (or very rarely two or three) of these nuclei comes to lie in close contact with the coenocentrum, and there grows (*figs. 35–37*) until it becomes many times its former

size and much larger than the nuclei found at earlier stages in the developing oosphere.

A study of oospheres after the second division shows the potential female nuclei in various stages of degeneration. They appear first to lose their chromatin network, the nucleoli and membrane persisting longest. Such degenerate nuclei can be found in abundance in the stages indicated. Frequently one or two rather large and apparently normal nuclei are found in an oosphere, which also has its one large nucleus in contact with the coenocentrum. In other cases, many very small nuclei are to be found in the ooplasm, and one large nucleus near the coenocentrum. These small nuclei range from the normal size to such small dimensions that only the nucleolus can be perceived with certainty. It would be impossible to recognize the smallest as nuclei, were it not that they are connected with undoubted nuclei by a series of complete gradations, and from the fact also that they lie in a cytoplasm remarkably clear and free from granules. Occasionally these extremely small nuclei are found in mitosis. Sometimes I have found groups of supernumerary nuclei, and often small ones are found in pairs, as though trying to fuse (*fig. 38*). Since this condition has never been found before the opening of the antheridial tube, it may represent the pairing of a supernumerary oospheric with a degenerate antheridial nucleus.

That these inclusions described as degenerate nuclei are really nuclei is established by their structure and behavior. That they do not result from the division of a fusion nucleus is shown by their presence when the male and female nuclei are found lying together, but still unfused in the same oosphere (*fig. 38*). The degenerate nuclei, as would be expected, are most numerous immediately after the second mitosis, while the one functional nucleus which lies near the coenocentrum is yet small. Before the opening of the antheridial tube nearly all have disappeared. There is convincing evidence that the oosphere is at first multinucleate (*figs. 30–32, 34*) and eventually uninucleate (*fig. 36*). There is no evidence to indicate

that any nuclei escape through the boundary into the periplasm, but they are seen in the ooplasm in all stages of degeneration.

The coenocentrum in *A. Tragopogonis*, as in *A. Bliti* and *A. Portulacae*, makes its appearance when the central mass of ooplasm is formed just before zonation (*figs. 27, 28*). It first consists of an area of cytoplasm which takes the orange G with great avidity, while the neighboring cytoplasm stains deeply with the gentian violet. A trifle later than zonation the coenocentrum is very highly developed, and appears in section as several zones of cytoplasm differing in density and stain reaction. The innermost area is coarsely vacuolate, and stains lightly with orange G. This region is surrounded by a narrower zone of dense granular cytoplasm, which is in turn encompassed by a less dense zone, and this finally by a broad zone of cytoplasm which stains more deeply with gentian violet. This condition is not greatly changed at the time of entrance of the primary oospheric nuclei represented in *fig. 30*. In later stages the outer zones are lost, and the innermost region assumes a characteristic homogeneous oily appearance and is quite spherical (*fig. 31*). About the time of the second mitosis the innermost region, all that remains of the coenocentrum, loses its clear appearance and becomes coarsely granular (*fig. 32*), and in its stain reaction shows the probability of nuclear contents. The vacuolate area seen in the center of very young coenocentra (*fig. 30*) is probably a reservoir for the reception of foods that are elaborated by the surrounding (presumably zymogenic) zone, which is in turn encompassed by typical trophoplasm. As the coenocentrum becomes older, these vacuoles, or rather globules of food stuffs in the protoplasm coalesce, and form the one central globule, which at first has a clear oily appearance (*fig. 31*), although it is not a true oil.

The coenocentrum possesses an attraction for the nuclei similar to that noted by Wager (1900) in *Peronospora parasitica*. Nuclei in greatly elongated condition, apparently moving toward the coenocentrum, give sufficient evidence of this (*figs. 30, 31*). As a result, several nuclei come into actual contact

with and even penetrate the coenocentrum (*figs. 32, 34*), and are thus found during all stages of the second mitosis. In some cases only one nucleus in mitosis is found thus attached; in others as many as three seemed to be so anchored, and probably, if the sphere could be viewed from all sides, as many as six or seven nuclei could be seen attached to the coenocentrum. The other primary oospheric nuclei are found lying free in the neighboring ooplasm in a similar stage of mitosis. It is not probable that any nuclei actually enter the coenocentrum, inasmuch as it maintains its homogeneous appearance until considerably later.

After the completion of the second mitosis, one small nucleus is found lying very close to the coenocentrum, possibly attached to it, although the evidence in *A. Tragopogonis* is not clear. Older stages show a larger nucleus. As the size of the nucleus increases, the coenocentrum becomes more granular (*figs. 35–37*), and loses its definite form, eventually appearing simply as a granular mass partially enveloping the female nucleus. As has been said, there is usually only one nucleus lying beside the coenocentrum after the completion of the second division, although several are in contact with it during this process. The oosphere when ready for fertilization contains one large nucleus which lies beside the remains of the coenocentrum. A few small degenerating nuclei, frequently present, are scattered throughout the ooplasm, which still maintains that fine-meshed alveolar structure that characterizes it after zonation.

Few phenomena of interest were observed in connection with the entrance of the antheridial tube. It has a much thinner wall, and is of more gelatinous nature, than that of *A. Bliti* or of *A. Portulacae*, and disappears more quickly after discharging its contents. It usually bears several degenerate nuclei as well as one or two that are larger and apparently capable of functioning. *Fig. 33* shows an unopened tube which contains a greater number, although in this case the oosphere clearly possessed only one functional nucleus. The rupture of the tube, accompanied

by the discharge of its contents, may occur at some distance from the female nucleus or nearly in contact with it. When the sperm nucleus is liberated it assumes an oval shape as it migrates toward the female (*fig. 36*).

The male is much the smaller when the nuclei first come in contact, although at this time it is larger than an ordinary vegetative nucleus. Fusion does not occur at once, but the nuclei lie together, imbedded in the remains of the coenocentrum. This period of rest must be of considerable duration, since the stage is found in great abundance, and the oosphere wall develops perceptibly at this time. During this period of quiescence the male nucleus enlarges until it eventually equals the female in size (*figs. 37, 38*) or nearly so. Fusion then occurs, both nuclei being in resting condition. An increase in the number of degenerating nuclei occurs in the oosphere simultaneously with the opening of the antheridial tube. This is quite evidently due to supernumerary antheridial nuclei which are clearly introduced (*fig. 33*). These nuclei, although some of them appear perfectly normal, at length degenerate and eventually dissolve, since only one nucleus is to be found when the fusion is complete. There is no evidence of a general fusion such as is described by Hartog (1891) and Golenkin (1900). This species presents a clear case of the presence of fertilized and unfertilized nuclei in the same cytoplasm, and illustrates the inability of the unfertilized nuclei to survive these conditions.

Before the accumulation of food advances far, the fusion nucleus divides, presenting a nuclear figure which is enormous in comparison with those in the oogonium and oosphere (*fig. 40*). As these divisions succeed one another, the nuclei decrease in size, so that those of the winter spore are about the size of the vegetative nucleus, and are probably about thirty or forty in number (*fig. 41*). Wager (1896) estimated the number in *A. candida* to be thirty-two. In ripening the spore exhibits those changes in wall and contents which characterize the other members of this group.

ALBUGO CANDIDA.

The result of the study of *A. Tragopogonis* suggested that the great variation in mode of fertilization seen in this genus might be explained in a way hitherto unsuspected. I have studied *A. candida*, therefore, comparing it stage for stage with the other species referred to in this paper. My observations essentially agree with those of Wager (1896), which were confirmed and extended by the research of Davis (1900). I have here only to present some further details, to make certain homologies clear, and in no radical way to alter the statements of these two writers.

The initial step in development consists of a differentiation of the ooplasm in the center of the oogonium, the nuclei in the meantime undergoing mitosis. One stage in this process is beautifully figured by Davis (1900, *fig. 2*), and a stage slightly older is herewith presented (*fig. 14*). It should be noted in these figures that the ooplasm is much more vacuolate than in the other known species (*figs. 3, 14, 28*). Particularly in the figure by Davis will it be seen that the ooplasm is separated from the periplasm by a rather broad irregular zone of very dense cytoplasm.

The conditions presented even in this early stage of oogenesis are markedly different from those of the other species. There is apparently no preliminary aggregation of cytoplasm into masses, but rather a simultaneous movement from the oogonial wall toward the center, leaving behind only slender threads of cytoplasm. It appears as though this movement occurs as a wave starting at the oogonial wall and moving so as to crowd the cytoplasm into the dense zone represented in the figures. When this condition obtains, the nuclei are approximately in metaphase, and it is clear that this represents the first nuclear division. Nuclei in metaphase and late anaphase may be seen in the same oogonium, but it is not probable that one nucleus undergoes more divisions than another, since the nuclei simultaneously pass through the stages of early prophase.

At a somewhat later stage, judging by the development of

the coenocentrum, the zone of dense cytoplasm represented in *fig. 14* (Davis 1900, *fig. 2*) disappears. The rudimentary oosphere consists then of uniformly distributed cytoplasm, together with the nuclei which have completed their mitoses (*fig. 15*). This condition is characterized by the well-developed coenocentrum, the presence of nuclei which are not in mitosis, and the central mass of dense, uniform, deeply staining cytoplasm which is suspended from the oogonial wall by delicate threads. Some of the nuclei now enter a second mitosis, and these may be found in various stages lying in this clearly marked central region. Those lying outside in the periplasmic region do not divide again, a distinction which is maintained in all species of Albugo.

Before the inception of this second mitosis, during its progress, or after its completion, the nuclei pass toward the periplasm (*figs. 15–17*) until all but one has left the ooplasm. The plasmoderma[3] is now formed, preceded by the first sharp differentiation of ooplasm from periplasm. This is immediately followed by the wall, a condition illustrated by Davis (1900, *fig. 4b*).

What stage in *A. candida* corresponds to zonation in the other species? Is it the stage represented by *fig. 14*, where the ooplasm is bounded by a dense layer of protoplasm; or later (*fig. 15*) when the oosphere is somewhat more sharply differentiated; or still later when the nuclei have left the central region and the plasmoderma is about to be formed? The stage shown in *fig. 14* has nothing in common with zonation except that the nuclei are in metaphase of the first mitosis. Zonation is chiefly characterized by that sharp differentiation of ooplasm and periplasm which precedes the plasmoderma. This character is absent here, as well as from the stage represented in *fig. 15*. A distinct differentiation does not obtain until all of

[3] The term *plasmoderma* has been suggested to me by Professor Strasburger as a desirable equivalent for the much-abused *Hautschicht*, which was originally intended for purely German usage. From its relation to cell activities (Noll 1888) and its characteristic kinoplasmic content the plasmoderma is to be regarded as a structure quite distinct from that designated by the term *ectoplast*.

the superfluous nuclei have left the ooplasm (Davis 1900, *fig. 3*). This is clearly the stage nearest to zonation in character, therefore, although it differs much from the zonation of the other species in general appearance. The absence ot preliminary protoplasmic aggregation precludes the early marshaling of the nuclei into the form of a hollow sphere, a phenomenon which is so characteristic of the zonation stage in *A. Bliti*, *A. Portulacae*, and *A. Tragopogonis*. It may be for this reason, also, that the formation of the plasmoderma is delayed.

The mode of development of the coenocentrum and its relation to the oospheric nuclei remain to be considered. The coenocentrum was first observed by Wager (1896) in *A. candida*, and its development has been more closely followed in the same species by Davis (1900). The structure was given its present name, and its nature and history were discussed to some extent, in my earlier paper on *A. Bliti* (1899). Wager (1900) further notes its presence in *Peronospora parasitica*, and observes that it has there an attraction for the one reentering female nucleus. I have already called attention to the same phenomenon in *A. Tragopogonis*. This attraction is strikingly exhibited in *A. candida*, and demands a somewhat detailed consideration.

A very early stage in development is shown by Davis (1900, *fig. 2.*) In a later stage the coenocentrum contracts, and a large globule appears at its center, much as in *A. Tragopogonis*, with the exception that the coenocentrum is very granular. The granules resemble the nucleoli of this species, both in size and stain reaction. They appear to enter the coenocentrum from the ooplasm in very early stages, and may be seen in great numbers in the adjacent region (*fig. 13*). The nuclei at this period are in mitosis, and it is quite usual to see two, three, or more spindles lying with one apex imbedded in the coenocentrum (*figs. 16, 18–20*). Their form is elongated before their attachment, and it is evident that the coenocentrum possesses an attractive influence. A similar attraction has been observed in *Peronospora parasitica* (Wager 1900) for resting nuclei, and by myself in *A. Tragopogonis;* but the phenomenon

of attraction is exhibited in *A. candida* during mitosis. It often results in a spindle nearly twice the normal length and proportionately narrower. Such cases are represented in *figs. 18–20*. The consequence of mitosis under these conditions is that several daughter nuclei remain anchored to the coenocentrum (*figs. 17, 21, 22*). Whether there is an organic attachment or merely an imbedding of a projection is impossible to say, but certainly these daughter nuclei protrude a long pseudopodium-like extension which penetrates the coenocentrum to a considerable depth. In late stages, as the nuclei pass to the periplasm, fewer are found attached to the coenocentrum, with the result that eventually only one remains. This nucleus enlarges greatly, and is often found lying in the cytoplasm in such a position as to suggest that it had been fixed while swaying to and fro on the stalk-like pseudopodium which attaches it to the coenocentrum. The migration and attachment of the nuclei to the coenocentrum seem inexplicable on any basis save that of chemotactic influence.

The growth of the nucleus while in contact with this structure, both in this species and in *A. Tragopogonis*, gives evidence that the coenocentrum functions as an organ of nutrition for the one surviving nucleus. In some respects the coenocentrum resembles a trophoplast, but the presence of granules, probably of kinoplasmic nature, renders the analogy less complete. It seems also to arise *de novo* in each oogonium, a character not in agreement with the theories most generally accepted. The evidence that such structures always arise from preexisting ones is not conclusive, however, and the work of Davis (1899) on Anthoceros may indicate that the chloroplast in that plant arises *de novo* in each sporophyte from the cytoplasm of the spore mother cells, although the author does not definitely draw that conclusion. If such be the case, the similarity between the coenocentrum and the chloroplast is closer. Evidence of high specialization in chloroplasts is given, moreover, in observations of Oltmanns on Coleochaete (1898), and of Davis on Anthoceros (1899), where the chloroplasts divide in advance of the nuclei, so

as to provide daughter structures equal in number to the nuclei which are to be formed. Considering the coenocentrum as a source of nutrition, the phenomenon of chemotaxis here as affecting the position of the nuclei is quite parallel to that exhibited so abundantly in animal cells, where the nuclei wander toward the source of nutritive supply (Korschelt 1889). The development of the pseudopodium-like structure also has its analogy in the animal cell, where the nucleus becomes amœboid or protrudes many pseudopodia in order to enlarge its absorptive surface (Korschelt 1889). In the nucleus under consideration the nature of the nutriment may render it more advantageous to penetrate by means of one sharp projection. While many plants possess cells having very irregularly shaped nuclei, *e. g.*, endosperm cells of Zea, epidermal cells of Allium, Hyacinthus (Zimmermann 1896), amœboid movements are much more rare in plants than in animals. Kohl (1897) by the action of asparagin has incited such movements in the nuclei of the cells of Elodea and Tradescantia, and considers them comparable with the phenomena observed by Korschelt (1889). This also throws light on the behavior of the nuclei in Albugo. Kohl considers with Korschelt that the amœboid movement stands in direct relation to the heightened exchange between nucleus and cytoplasm.

The coenocentrum may in a sense be likened to some of the so-called yolk nuclei, or *Dotterkern* of animal eggs. In *A. Bliti* (Stevens 1899, *fig. 69*) and in *A. candida* (Davis 1900, *fig. 2*) there is slight indication of radiate structure which somewhat resembles the figures of Munson (1898) illustrating the yolk nucleus of Limulus. The structures agree in having nutritive functions. In certain cases, as in Limulus and the newt (Jordon 1893), the yolk nuclei seem to develop directly from the cytoplasm, and in this further resemble the coenocentrum. A comparison of these structures emphasizes the fact that protoplasm in diverse organisms under certain conditions may become similarly differentiated for the performance of particular functions.

Concerning the antheridial tube there is but little to add.

Both Wager (1896) and Davis (1900) assert that it bears one functional nucleus, but they figure other nuclei in the neck of the tube, and Davis suggests that occasionally more than one nucleus from the antheridium may enter the ooplasm. *Fig. 26* shows a case where the tube clearly contains one large male nucleus in a mass of dense cytoplasm, and another nucleus lying in such a position that it must be set free were the tube to open. It is also common to find more than two nuclei in the oosphere just after the opening of the tube and before fusion. In such cases it is evident that there is a supernumerary nucleus, but whether it is derived from the antheridium or the oosphere is not always plain. Such conditions as those shown in *fig. 17* render it probable that sometimes two or even more female nuclei might survive. The supernumerary nuclei would then be found near the coenocentrum (*fig. 23*). Occasionally the one supernumerary nucleus lies free in the ooplasm at a considerable distance from the coenocentrum, and is probably of antheridial origin. It is quite conceivable, and it may occasionally happen, that two functional nuclei are derived from each organ, and that a multiple fusion of nuclei may occur even in *A. candida*.

Figs. 24 and *25* represent two stages in the division of the fusion nucleus. The strongly thickened fibers and their contorted condition agree well with the view of Drüner (1895) that the spindle poles are separated by a push due to an elongation of the spindle, not by a pull from a region external to the nucleus, nor by cytoplasmic streaming. The division is of quite the same type as that exhibited before fusion, an agreement that is particularly evident in anaphase, so characteristic in both this and other species.

A fuller study of the species has developed one essential point somewhat at variance with previous observations. I believe I have shown that there is no time when the central region in *A. candida* is entirely devoid of nuclei, and that very early there is an attractive force exhibited by the coenocentrum which results in a retention of one nucleus while the others are

ALBUGO PORTULACAE.


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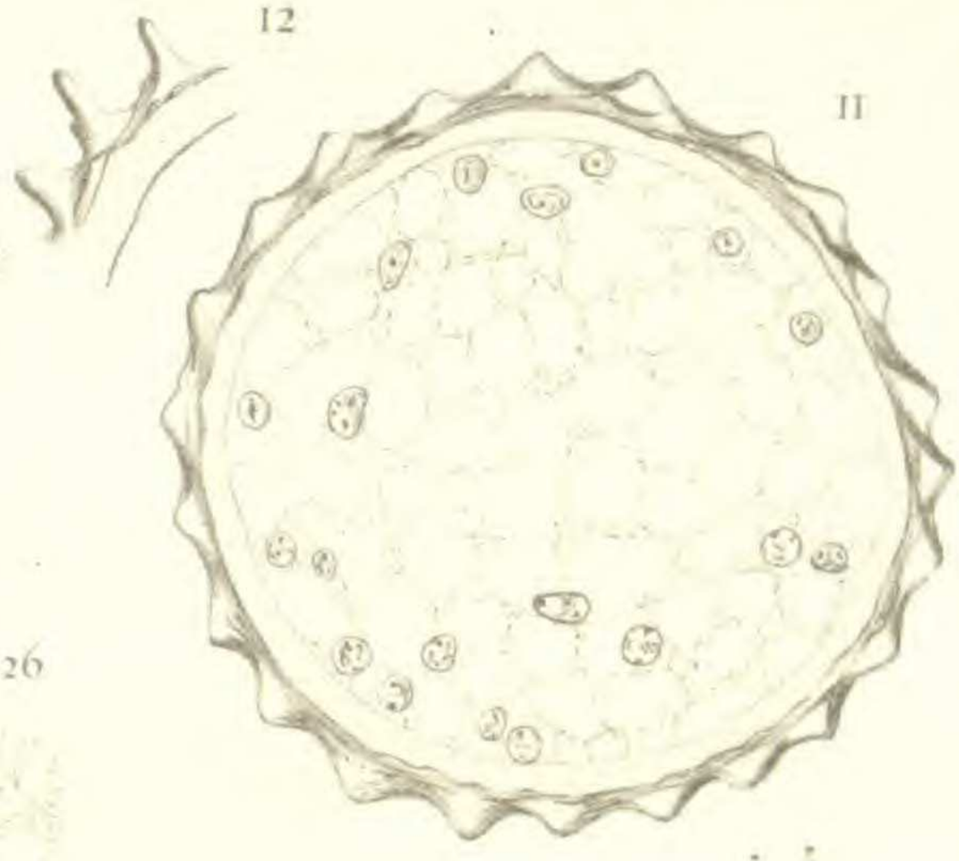
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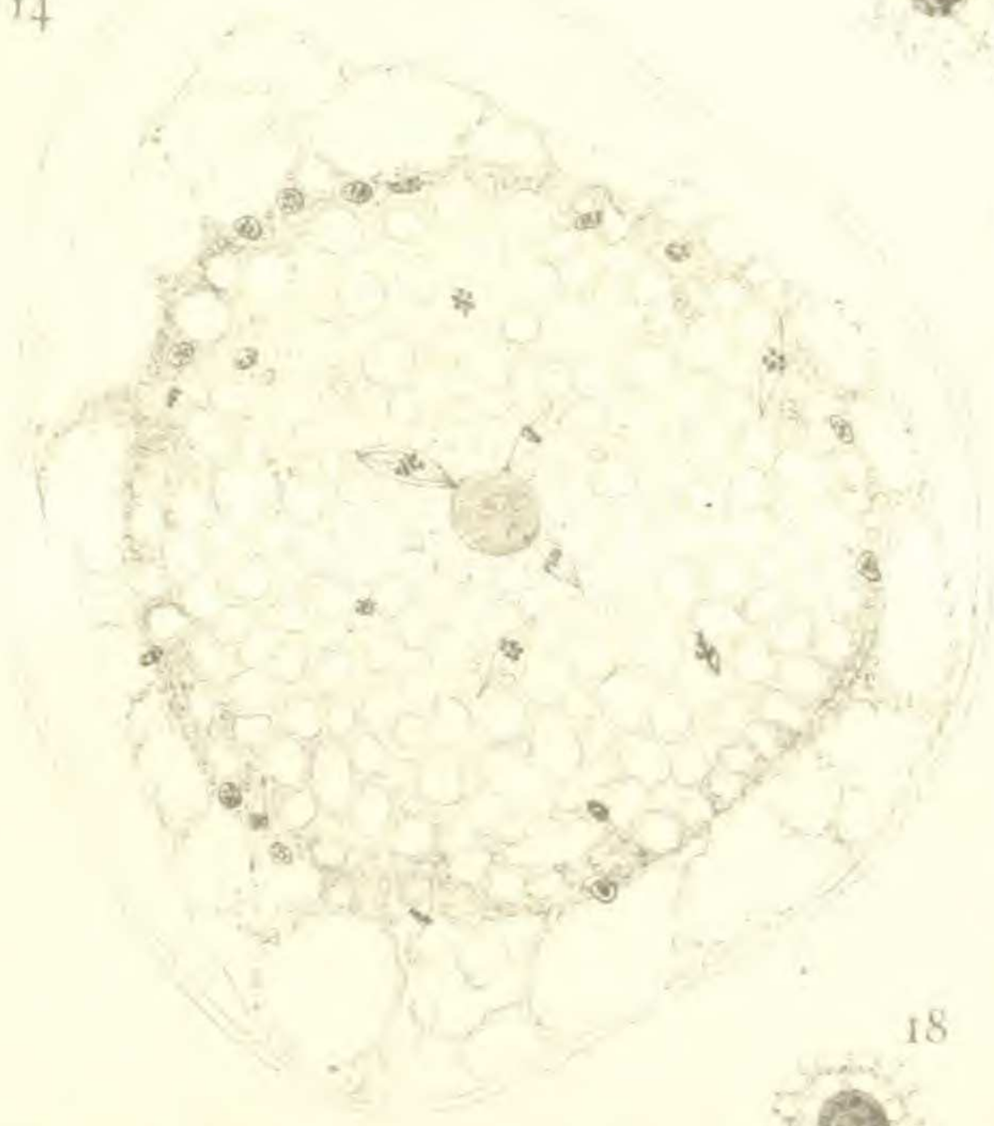
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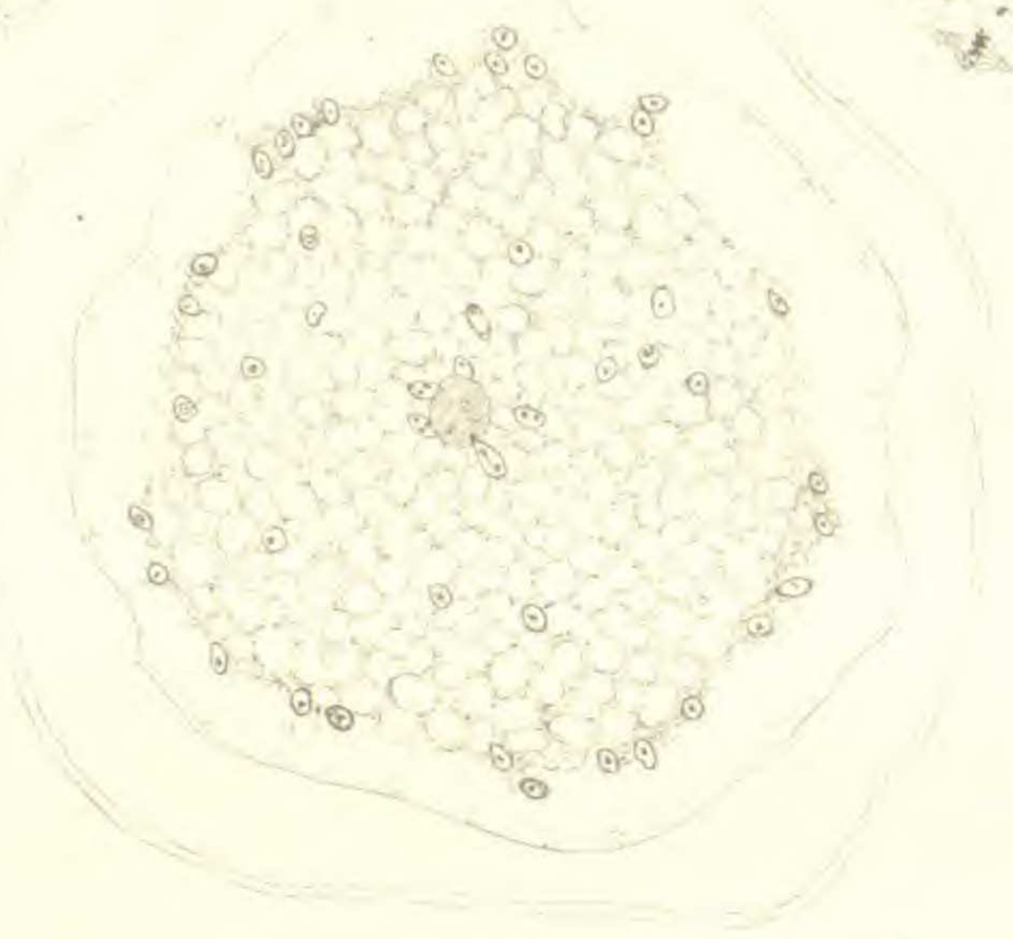
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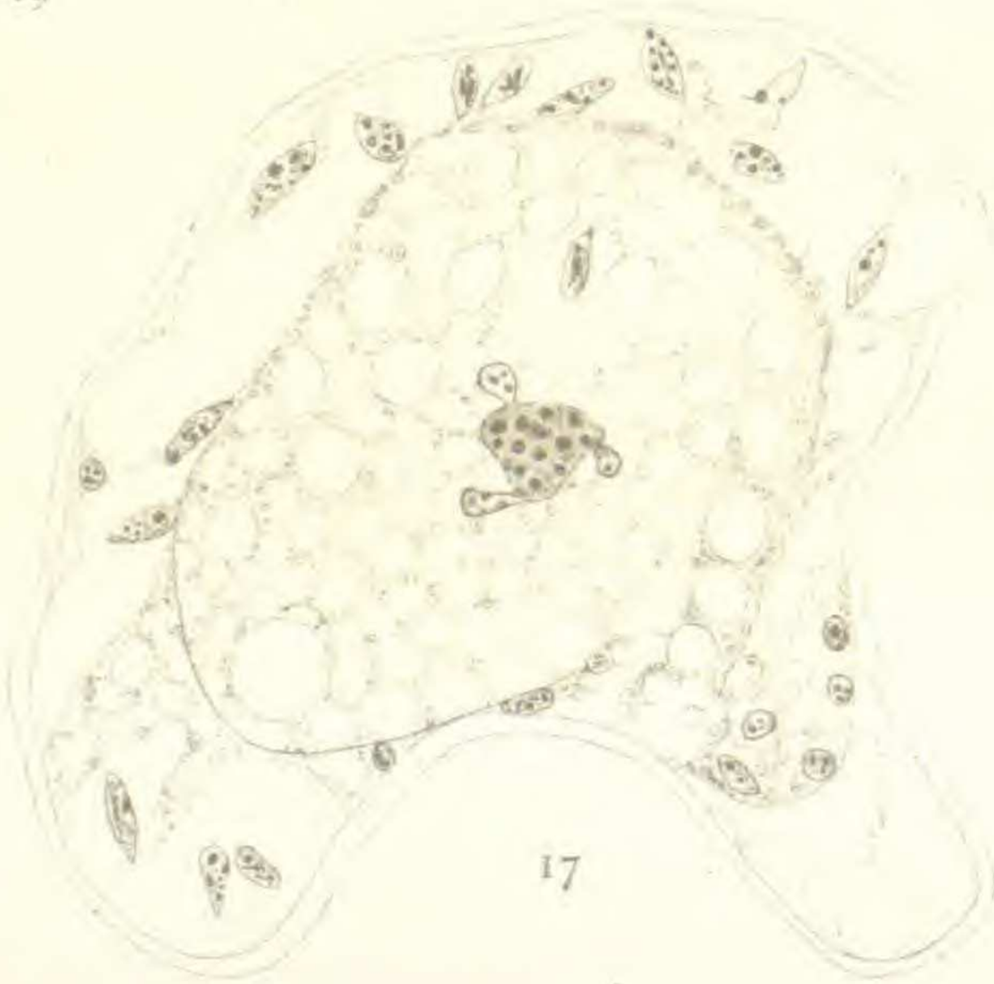
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